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Effects of ADP-ribosylation of GTP-binding protein by pertussis toxin on immunoglobulin E-dependent and -independent histamine release from mast cells and basophils.

Saito H, Okajima F, Molski TF, Sha'afi RI, Ui M, Ishizaka T.

Pretreatment of rat peritoneal mast cells, human basophils, bone marrow-derived mouse mast cells (BMMC) and mouse mast cell line PT-18 cells with 1 microgram/ml pertussis toxin (PT) failed to inhibit immunoglobulin E (IgE)-dependent histamine release from the cells. In BMMC and PT-18 cells, even 20-hr incubation of the cells with 1 microgram/ml PT, which ADP-ribosylates more than 97% of 41 kDa, alpha-subunit of Ni in the cells, failed to affect the IgE-dependent release of histamine or arachidonate. The results indicate that GTP-binding protein, Ni, is not involved in the transduction of triggering signals induced by cross-linking of IgE receptors. In contrast, pretreatment of rat mast cells with 1 ng/ml to 0.1 microgram/ml PT for 2 hr inhibited histamine release induced by compound 48/80 in a dose-dependent manner. A similar pretreatment with PT inhibited thrombin-induced histamine release from BMMC and N-formyl-L-methionyl-L-leucyl-L-phenylalanine-induced histamine release from human basophils in a similar dose-dependent fashion. However, even 20 hr of incubation of sensitized BMMC with 1 microgram/ml PT failed to inhibit either thrombin-induced or antigen-induced breakdown of phosphatidylinositides (PI), i.e., the formation of inositol triphosphate and diacylglycerol, Quin-2 signal, and the release of arachidonic acid. The results indicate that the inhibition of thrombin-induced histamine release by PT-treatment is not due to the inhibition of PI-turnover, and that Ni is not involved in thrombin-induced or antigen-induced (IgE-dependent) hydrolysis of phosphatidylinositides in mast cells.

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Thirty years of stimulus-secretion coupling: From Ca^{2+} to GTP in the regulation of exocytosis

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Abstract — Calcium, initially considered as the universal link between receptor stimulation and the onset of exocytosis in secretory cells, is now recognised as only one of a number of intracellular activators. In cells of haematopoietic origin (including mast cells), the key activator is one or more GTPases. Cells of this class, stimulated with GTPγS can undergo exocytosis in the effective absence of Ca^{2+}. A number of GTP-binding proteins that mediate exocytosis (G_E) have been proposed but the best evidence supports roles for members of the Rho family of monomeric GTPases and for βγ-subunits derived from G_{i3}. While preactivated Rac and Cdc42 can induce secretion from permeabilised mast cells in the absence of a guanine nucleotide βγ-subunits only act to enhance the secretion induced by other GTP-binding proteins (likely to be members of the Rho family of monomeric GTPases). Further work is required to identify downstream effectors activated by these GTP-binding proteins and to show how they interact with the SNAP and SNARE isoforms known to be present in these cells. © 2000 Société française de biochimie et biologie moléculaire / Éditions scientifiques et médicales Elsevier SAS

stimulus-secretion coupling / Ca^{2+} / GTP / regulation of exocytosis

1. Introduction

There has been an enduring interest in the regulation of secretory processes. A central role for Ca^{2+} first became apparent in the 1960s, particularly with the work of W.W. Douglas. He showed that the secretion of catecholamines from adrenal chromaffin cells [1] is dependent on the presence of Ca^{2+} in the extracellular medium. A role for Ca^{2+} is evident also in cells which respond directly to stimulatory ligands and do not depend upon a membrane depolarisation. Among these are the cells of the immune system, in particular the mast cells (responding to ligands causing the cross-linking of receptors for immunoglobulin E (IgE)), neutrophils (responding to bacterially-derived formylmethionyl peptides, complement factors, aggregated IgG etc.), eosinophils (responding to cytokines). All of these cells, and also the neuroendocrine and endocrine cells, undergo secretion in response to treatment with Ca^{2+}-carrying ionophores, (*Streptomyces*-derived products which convey Ca^{2+} through the lipid bilayer of membranes and thus act to raise the concentration of intracellular Ca^{2+}). Based on this wide experience and observations on several other cell types, and bearing in mind the established role for Ca^{2+} as the regulator of muscle contraction, he proposed that Ca^{2+} comprises the key link in a train of events which he called stimulus-secretion coupling [2].

At that time, the role of Ca^{2+} as a link between receptor activation and exocytosis was widely perceived in terms of a physical interaction between the doubly charged Ca^{2+} ion and the negatively charged surfaces of the fusing membrane surfaces [3]. Thirty years later, a place for Ca^{2+} at some point in the stimulus-secretion pathway remains assured but it is now apparent that it interacts with specific high affinity Ca^{2+} binding proteins. What is not so clear is what these Ca^{2+} binding proteins actually do [4]. More than this, it has become apparent that for some secretory cells and under some conditions, it is possible to induce secretion in the effective absence of Ca^{2+} (intracellular Ca^{2+} concentration < 10^{-8} M) and others in which Ca^{2+} even acts to inhibit, rather than to promote secretion.

2. Exocytosis without elevation of cytosolic Ca^{2+}

Nearly all the proteins implicated by the SNARE hypothesis in exocytosis are universally expressed, and it is widely understood that all secretory cells employ the same basic mechanism to direct the fusion of the plasma membrane with internal vesicular membranes. This statement includes, of course, the class of myeloid cells and other cells of haematopoietic origin in which the place of Ca^{2+} as the primary regulator is now being challenged. In

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particular this statement encompasses the mast cells with which our work has been primarily concerned. These express an isoform of synaptotagmin (probably synaptotagmin III [5]) which is a potential Ca^{2+} sensor [6]. Yet, in spite of all this, with the development of permeabilized cell preparations and also through patch clamp electrophysiology, it has become evident that the role of Ca^{2+} in the ultimate stages of exocytosis in these cells is secondary to that of another activator, namely GTP [7–9]. Indeed, there are circumstances under which exocytosis can be elicited even when Ca^{2+} is effectively excluded from the system [8, 10, 11]. In neutrophils permeabilized (with Sendai virus), we found that the extent of secretion due to GTPγS was actually enhanced as the concentration of Ca^{2+} was suppressed by the application of high concentrations of EGTA or BAPTA (Ca^{2+} chelators). It became clear that the determining events in this class of cells involve the activation of GTP-binding proteins. Our attention has therefore turned to the identification of the particular GTP-binding proteins that convey the signals to the secretory machinery, the pathways through which these GTP-binding proteins are activated and the immediate consequences of their activation.

3. Two pathways leading to exocytosis

In mast cells, the agents which stimulate the secretory reaction fall into two main classes. Specific antigens (allergens) act indirectly to cross-link the cell surface immunoglobulin receptors [12]. They do this by binding to IgE, which is tightly bound to high affinity Ig receptors (IgεRI) situated in the plasma membrane of mast cells and basophils. In fact, not only antigens, but any ligand that acts to cross-link the IgE receptors can act as a stimulus. Thus, lectins (which bind to polysaccharide chains exposed on the receptor), anti-receptor antibodies or oligomerized Fcε can all stimulate secretion. The cross-linking reaction initiates a series of phosphorylations on tyrosine residues [13] causing the recruitment and activation of phospholipase-Cγ and consequent generation of IP_3 and diglyceride. This leads to the elevation of cytosol Ca^{2+} and activation of protein kinase C [14]. The second class of activators comprises the so-called basic secretagogues. These include natural agents such as the wasp venom peptide mastoparan as well as the synthetic compound 48/80. Agonists of this class, so-called receptor-mimetic agents, appear to bypass conventional receptors and to activate GTP-binding proteins directly [15]. They can induce exocytosis without activation of PLC and without elevation of cytosol Ca^{2+} [16, 17]. This pathway has been implicated in a number of inflammatory conditions. Finally, secretion from mast cells can also be induced by application of Ca^{2+}-carrying ionophores which convey Ca^{2+} ions directly into the cytosol without the involvement of any receptors or associated processes [18, 19]. Secretion due to all these agents can be prevented by application of metabolic inhibitors resulting in the depletion of ATP.

The development of techniques allowing direct manipulation of the composition of the cytosol, by permeabilization or by application of a patch pipette, has provided the most direct evidence for the role of GTP and GTP-binding proteins. As shown in *figure 1A*, the poorly hydrolysable GTP analogue, GTPγS, can elicit secretion from permeabilized cells in the effective absence of Ca^{2+} (10^{-9} M). In many other systems (e.g., neuronal, neuroendocrine and endocrine cells) elevation of intracellular Ca^{2+} is the major or at least a sufficient stimulus to secretion. However, while application of Ca^{2+} in the range of 1–10 μM to permeabilized mast cells can elicit secretion, this remains dependent on the presence of GTP. This is made clear in *figure 1B* which shows that secretion (measured as released hexosaminidase) increased as the concentration of GDP was elevated in the range 10–30 μM. As the concentration of GDP was then further increased from 30 μM to 1000 μM the secretion was inhibited. The positive effect of GDP in the lower concentration range is, of course, due to its conversion to GTP by nucleoside diphosphate kinases, which remain active in the permeabilized cells [20]. Although dispensable, Ca^{2+} invariably acts to enhance the level of guanine nucleotide-induced secretion. Also, pertinent to the discussion presented below, there are conditions in which Ca^{2+} is obligatory, particularly when the stimulus is applied after a period of time following permeabilization. (Note: the late application of a stimulus to permeabilised cells is applied as a procedure that permits introduction of exogenous proteins.)

Just as the presence of Ca^{2+} is not an absolute requirement for exocytosis from permeabilised cells, nor is ATP [7] and from this it follows that a phosphorylation reaction cannot be a determining step in the final stages of the stimulus-secretion pathway (meaning the steps following the activation of GTP-binding proteins). The sensitivity of intact cells to metabolic inhibitors can be ascribed to the requirement for ATP in the tyrosine phosphorylation reactions leading to activation of PLCγ and for maintaining the pool of GTP through the nucleoside diphosphate kinase reaction. On the other hand, just as with Ca^{2+}, the system becomes ATP-requiring when the stimulus to secrete is applied after a delay of some minutes following permeabilization, allowing proteins to leak from the cells. This gives the clue that a phosphorylation state, not a reaction is obligatory. Such a state is likely to involve both protein and lipid phosphorylations, as outlined below. Among other important functions linked to the phosphorylation state is the maintenance of a high effective affinity of the secretory process for Ca^{2+} [21].

In intact cells, it is the mode of activation that determines which GTP-binding proteins are involved. Thus the stimulation of mast cells by the polycationic, receptor-

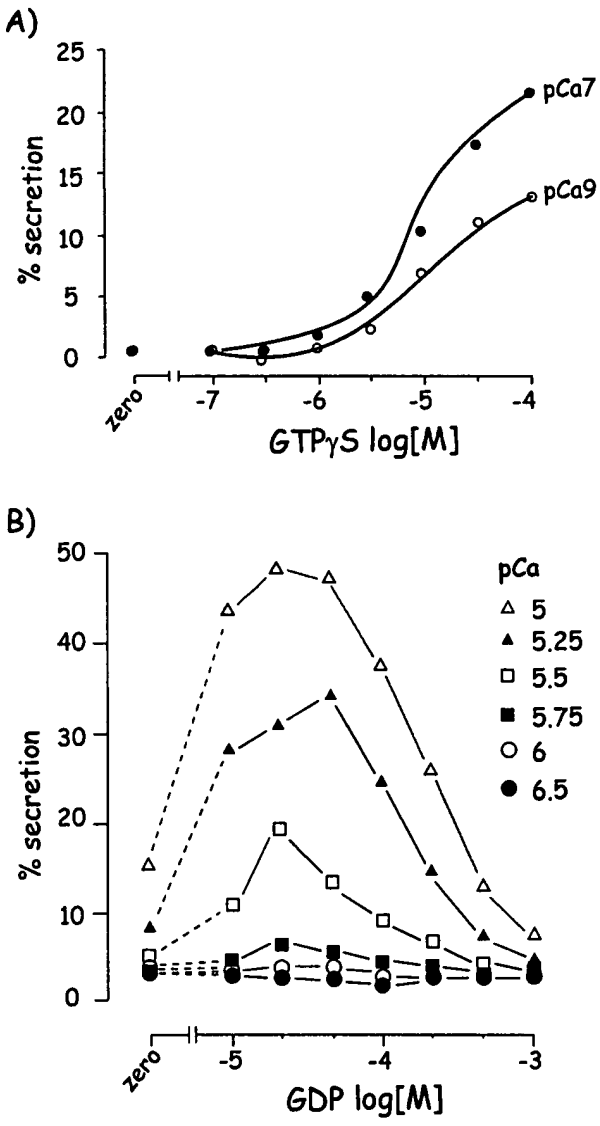

Figure 1. The exocytotic mechanism of mast cells is dependent on guanine nucleotides and can occur in the effective absence of Ca^{2+}. **A.** Secretion from permeabilized mast cells induced by GTPγS in the effective absence of Ca^{2+}. Mast cells were incubated in the presence of streptolysin O together with MgATP (1 mM), calcium (buffered with EGTA at the concentrations indicated) and GTPγS. After 10 min the cells were sedimented and the supernatant was assayed for released hexosaminidase. **B.** Activation and inhibition of secretion from permeabilized mast cells by GDP. Mast cells were incubated in the presence of streptolysin O together with MgATP (1 mM), calcium (buffered with EGTA at the concentrations indicated) and GDP as indicated. After 10 min the cells were sedimented and the supernatant was assayed for released hexosaminidase.

mimetic agonists is prevented by pertussis toxin pre-treatment, while activation by agents that cross-link the receptors for IgE is unaffected (see Note added in proof). Yet depletion of cellular GTP (by pre-treating cells with ribavirin or mycophenolic acid, inhibitors of IMP dehydrogenase) prevents secretion due to all forms of stimulation [22, 23]. Importantly, this includes stimulation by Ca^{2+} ionophores and it follows that we are looking for at least two GTP-binding proteins in the stimulus-secretion pathway. An obvious possibility that has been considered is that the activation of a GTP-binding protein causes the activation of phospholipase C, releasing inositol 1,4,5-trisphosphate (IP_3) and so causing the release of Ca^{2+} from internal stores. However, the application of the aminoglycoside antibiotic neomycin, sufficient to cause full inhibition of phospholipase C, was shown to be without significant effect on secretion from the permeabilized cells [24]. Thus, not only is the role of Ca^{2+} indirect, but phospholipase C also, plays no essential role in the exocytotic mechanism of these cells. From these and other experiments [16], it also became apparent that the site of action of Ca^{2+} (and of course, phospholipase C) is situated upstream of the action of the GTP-binding proteins [8].

The first clues hinting at the identity of a heterotrimeric G-protein in the pathway initiated by the receptor-mimetic agents came through the application of reagents (peptides and antibodies) interacting with the C-termini of the α-subunits of G_i proteins to permeabilized mast cells (and RBL-2H3, a mast cell related line) [25]. Only those reagents directed to α_{i3} caused inhibition: similar reagents having specificity for α_{i2} (strongly expressed in mast cells) were without effect. As a result, G_{i3} was proposed to have the properties of a G_E, a GTP-binding protein that mediates exocytosis [25]. On the other hand, an important pointer for a role for monomeric GTP-binding proteins of the Rho family was the observation that RhoGDI (a protein that solubilizes and sequesters GTPases of this class), applied to permeabilized cells, inhibits GTPγS-induced secretion [26, 27].

Inhibition by RhoGDI clearly demonstrates that one or more of the Rho-related GTPases must play a central role in the exocytotic mechanism and the generality of this pathway is hinted at by the finding that RhoGDI also inhibits secretion from permeabilized eosinophils *(figure 2)*. In particular, Rac and Cdc42 are strong candidates [28]. Rac first emerged as a possible G_E protein as a result of the fractionation of brain cytosol. We used as an assay the ability of fractions to extend the period during which mast cells remain responsive to stimulation by GTPγS following permeabilization. This style of experiment, the 'run-down' procedure, allows the introduction, by diffusion, of exogenous proteins into the permeabilized cells. Due to the simultaneous efflux of proteins of the cytosol, the propensity of the cells to remain responsive to stimulation declines (typically over a period of about 20 min). A 43 kDa protein (FOAD II, factor of activation of degranu-

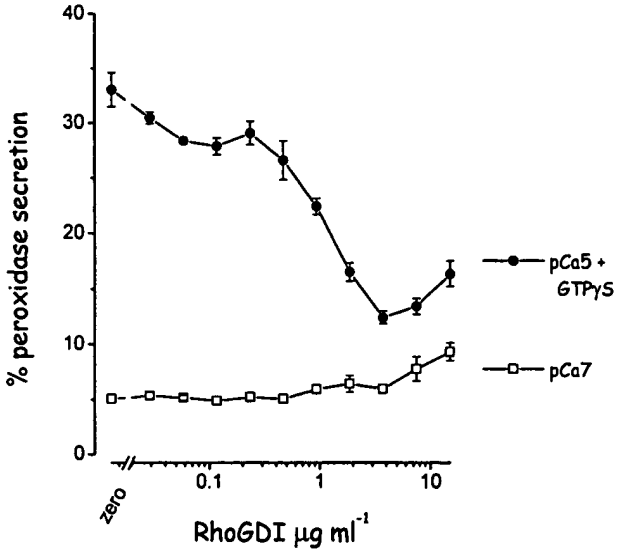

Figure 2. Inhibition of secretion from permeabilized eosinophils by RhoGDI. Guinea pig eosinophils were permeabilized with streptolysin-O in the presence of low calcium (pCa8), ATP and RhoGDI at the concentrations indicated and incubated for 10 min. They were then stimulated to secrete by addition of GTPγS and calcium (pCa5) and incubated for a further 10 min. Secretion was measured as the release of eosinophil peroxidase.

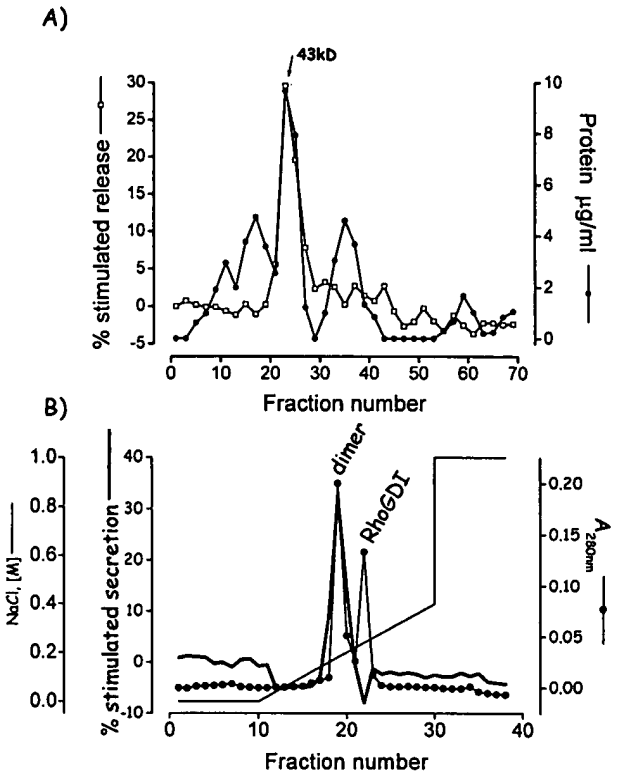

Figure 3. Resolution of FOAD II into two components. Chromatography of partially purified protein from bovine brain by gel filtration on G75 Sephadex. The active material eluted as a single peak at 43 kDa. Gel electrophoresis revealed that it was composed of two components having a relative molecular mass of 22 and 28 kDa. The smaller component bound GTP. Anion exchange chromatography on MonoQ separated FOAD II into its separate components. The 28 kDa protein was found to inhibit secretion in the run-down assay and sequence analysis revealed it to be RhoGDI. The activating protein is the dimeric complex of RhoGDI with Rac.

lation) was identified that supports secretion (extended the period of responsiveness to stimulation) *(figure 3)*. Electrophoretic analysis indicated that this is composed of two components, one of which (22 kDa) binds guanine nucleotides. Separation of the heterodimer by cation exchange chromatography yielded two components, one of which (28 kDa) proved to be an inhibitor. Sequence analysis then revealed this peptide to be RhoGDI and the GTP binding component to be Rac-I. While native Rac added to the permeabilized cells was without effect (probably due to its insolubility), its complex with RhoGDI (FOAD II) was found to support secretion [27].

4. Secretion induced by activated GTPases

In contrast to the native protein, application of recombinant Rac or Cdc42, preactivated by attachment of GTPγS, induces exocytosis in the presence of calcium [28]. Significantly, for secretion induced by these GTPases, the presence of free GTPγS is dispensable, though when provided it enhances the extent of secretion giving the clue that other activating GTP-binding proteins are present. Because the recombinant proteins are soluble, lacking any post-translational lipid modification, they do not have to be presented as heterodimeric complexes with RhoGDI. They are able to find their way to their appropriate locations in the cell interior.

An encouraging pointer for future work is that Rac containing the substitution Y40C (in the so-called effector domain) retains its capacity to cause exocytosis. This mutant is unable either to interact with the Ser/Thr kinase p65PAK or to activate the JNK MAP kinase pathway, both known effector systems of the Rho family. By contrast, another effector domain mutant, F37A, is inactive. This mutation is understood to react with those effectors that contain a so-called CRIB (Cdc42/Rac interaction binding) sequence; it is unable to interact with the Ser/Thr kinase p160ROCK, but retains the ability of the wild-type to induce rearrangements of the cytoskeleton [28, 29]. Hopefully, other mutants in the effector binding domains of these GTPases will provide further clues to the identity of their immediate downstream partners.

5. βγ-subunits enhance secretion induced by GTPγS

The activating role for G_{i3} in the pathway initiated by the receptor-mimetic agents was based on the use of reagents that prevent the dissociation of the heterotrimer into its active components [25] and so it was unclear whether the signal is transmitted through α_{i3} or βγ. In order to resolve this problem, we set out to test the effect of isolated α_{i3} and βγ-subunits provided to the permeabilized cells in the run-down procedure. It became apparent that α-subunits are without discernible effect on secretion induced by GTPγS. On the other hand, βγ-subunits, both purified and recombinant, act to enhance the extent of secretion [30]. However, unlike the monomeric GTPases Rac and Cdc42, the βγ-subunits are unable to induce secretion by themselves. It follows that they operate in conjunction with other GTP-binding proteins; obvious candidates must be Rac and Cdc42.

Several other proteins have been proposed as possible candidate G_Es, GTP binding proteins that mediate exocytosis. Microinjection of the persistently activated Ras oncogene product was found to induce the morphological changes characteristic of degranulation following a 4-h incubation [31]. The idea that activation of endogenous Ras might be a component of the normal stimulus-secretion pathway was never very plausible and, with hindsight, it seems possible that the effect might have been due to the induction of protein synthesis and the conversion of the cells into constitutive secretors.

The possibility that Rab3A might be a regulator of secretion came from experiments in which peptides derived from the effector loop of this protein appeared to induce secretion in patch-clamped mast cells [32]. It was subsequently shown that this peptide also suppresses cAMP levels and induces Ca^{2+} transients in mast cells [33], raising the possibility that its mode of action is non-specific. Additionally, peptides with modified sequences were also found to cause fusion between pancreatic zymogen granules and plasma membranes [34]. The possibility of a role for Rab3A at some point in the stimulus-secretion pathway was indicated by inhibition of IgE-induced secretion by transfection of a dominant negative mutant, albeit in RBL-2H3 cells. Since secretion from the transfected RBL cells could still be induced by GTPγS following permeabilization it appears that the block must be upstream of the site of action of G_E [35]. Recent work has demonstrated that the main isoform of Rab3 present in mast cells is Rab3D and that this protein translocates to the secretory granules when the cells are stimulated [36]. Clearly, further work is required to determine whether Rab3D has any direct effect on exocytosis.

Another GTP-binding protein that has been suggested to act as a regulator of exocytosis is ARF, based on its ability to prolong the run-down period in permeabilized HL60 cells [37]. We have failed to demonstrate any such effect in mast cells (O'Sullivan and Gomperts, unpublished observations). The lesson to be heeded from this, and many other reports, is that the behaviour of cell lines is often only very distantly related to what actually happens in real cells.

6. Downstream targets of βγ-subunits

In considering the possible downstream targets for the βγ-subunits, an obvious candidate is PKC, activated through the products of PLCβ [38]. However, the enhancement of secretion due to βγ-subunits remains manifest even under conditions of maximum activation by phorbol ester [30]. It follows that in addition to the activation of protein kinase C, the signal due to βγ-subunits must also be relayed through a second, independent pathway. This is likely to involve proteins having PH domains. We showed that reagents known to impede the interaction of βγ-subunits with PH domains (phosducin and the C-terminal peptide of the β-adrenergic receptor kinase 1, βARK_1) act to inhibit secretion induced by GTPγS and also to abrogate the enhancement due to exogenous βγ-subunits. The βARK_1 PH domain, however, also interacts with polyphosphoinositides. However, mutants that interact preferentially with polyphosphoinositides (rather than βγ-subunits) remain inhibitory in the run-down assay. Thus, the phosphorylation state determining the competence of mast cells to undergo secretion is likely to involve both proteins and inositol lipids.

Support for this proposal comes from our recent experiments with phosphatidylinositol transfer proteins (PI-TP) that mediate the transfer of phosphoinositides to the cell membrane. We find that these too act to delay the progress of run-down (i.e., they support secretion). Conversely, reagents that interfere with this process (mutant PI-TP) or that block the head group of the inositol phospholipid (neomycin) accelerate run-down (inhibit secretion) [39]. It would appear that any procedure that acts to maintain the membrane complement of polyphosphoinositides extends the period of time following permeabilization, during which the cells retain their sensitivity to stimulation by guanine nucleotides and Ca^{2+}.

Although, as already remarked, phosphorylation is not a determining step in the pathway to exocytosis, the system does become sensitive to ATP as run-down proceeds. The concentration required, (in the range 25–50 μM), should be sufficient to support phosphorylation by PKC [40]. Also, it should be sufficient to support phosphorylation by PI 4-kinase [41] and some PIP 5-kinases [42, 43]. Alternatively, or additionally, a PI 3-kinase could be involved. However, although an isoform regulated by βγ-subunits has been implicated in the secretory pathway of neutrophils [44], we have been unable to perceive any effect of compounds such as the inhibitors, wortmannin and LY294002 upon GTPγS induced secretion from permeabilized mast cells [30].

7. Linking the two pathways of activation

It will be important to show that the enhancement of secretion induced by βγ-subunits is prevented by RhoGDI and also if it still occurs in the absence of free guanine nucleotide when the stimulus is provided by pre-activated Rac and/or Cdc42. This would confirm that the βγ-subunits are linked to the pathway of activation mediated by these proteins and not through further GTPases. The report that Vav acts as a guanine nucleotide exchange protein [45], linking the effects of the Src and Syk/ZAP family of non-receptor tyrosine kinases (e.g., Lyn and Syk) to the activation of the Rho-related GTPases, provides the basis of an activating pathway from the receptor for IgE [46–48]. The basic plan is illustrated in *figure 5*.

Since the enhancement of exocytosis by βγ-subunits depends on the presence of an activating guanine nucleotide, it follows that they must activate or regulate another G-protein, either directly or, more likely, indirectly. In particular, members of the Dbl family (mammalian homologues of the yeast guanine nucleotide exchanger, Cdc24) operate as regulators for a number of Rho-family GTPases. Adjacent to the Dbl homology domain (DH) which comprises the nucleotide exchange activity, these proteins also contain at least one PH domain which could render them amenable to regulation by βγ-subunits. There is good evidence that these PH domains mediate recruitment of the proteins to the membrane through interaction with lipids, βγ-subunits or isoforms of PKC [49].

Having established which monomeric GTPases are the physiological regulators of exocytosis we are in position to consider the identity of their downfield effectors. There is the possibility to be considered that the actions of the Rho-related GTPases are directly mediated by the cytoskeleton. This seems to be unlikely. Although it undergoes extensive reorganisation in response to the activation of these GTPases it appears that neither the extent and nor the rate of secretion from mast cells is affected by manipulations (e.g., application cytochalasin) designed to affect the organisation of the cytoskeleton [50].

More pertinently, it should be possible to address the important questions regarding the relationship between GTPase activation and the SNARE proteins and their role in the induction of exocytotic fusion. In preliminary experiments *(figure 4)* we have found that botulinum toxin C1 (BontC1) accelerates run-down (in eosinophils); BontA and BontB appear to be without effect. This suggests that a syntaxin is a component of the fusion complex. In addition, a recent report describes the role for SNAP-23 (not cleaved by BontA and BontE) in mast cells [51].

8. Conclusion

Contributions from other groups investigating T-lymphocytes [52, 53], platelets [54, 55], and our own

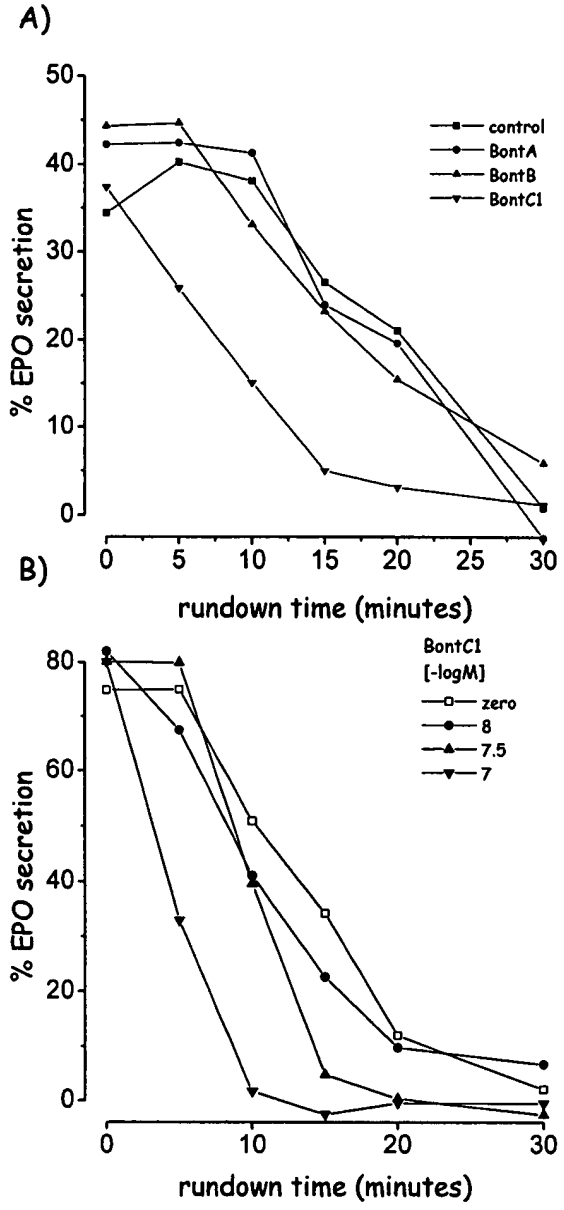

Figure 4. Inhibition of secretion from permeabilized eosinophils with botulinum C1 toxin. Guinea pig eosinophils were permeabilized with streptolysin O in the presence of low calcium (pCa8), ATP and botulinum toxins A, B and C1 (10^{-7} M), all previously activated by reduction. At the times indicated the cells were stimulated to secrete by addition of GTPγS and calcium (pCa5) and incubated for a further 10 min. Secretion was measured as the release of eosinophil peroxidase. Guinea pig eosinophils were permeabilized with streptolysin O in the presence of low calcium (pCa8), ATP and botulinum toxin C1 at the concentrations indicated. After 10 min they were stimulated to secrete by addition of GTPγS and calcium (pCa5) and incubated for a further 10 min.

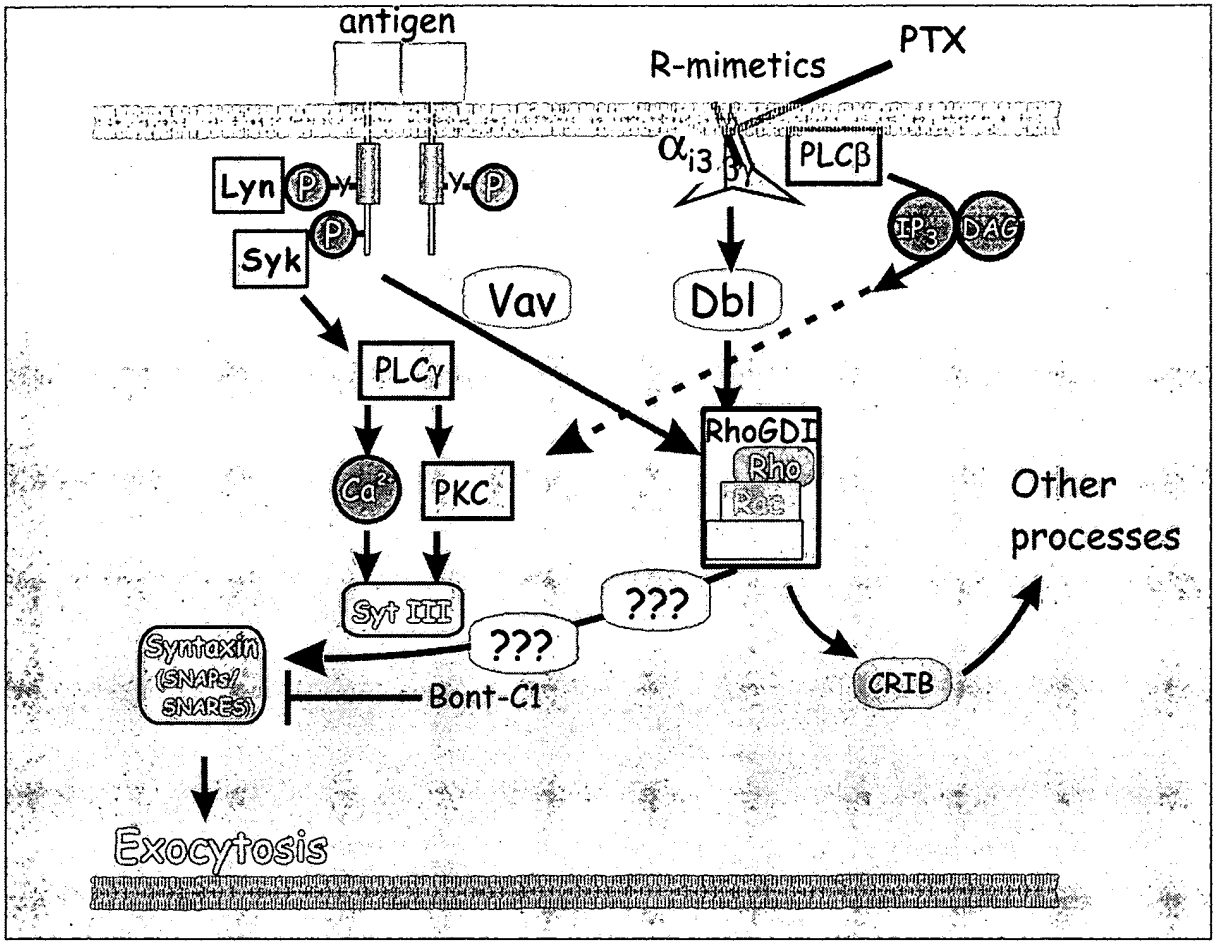

Figure 5. Scheme summarising lines of communication between the main components of the stimulus-secretion pathway in mast cells. For details, see text. Note that the identity of those items lacking a strong outline are inferred, not being based on direct experimental evidence.

work on eosinophils [56–58] and neutrophils [11], lead us to understand that the regulation of secretion by GTP-binding proteins is a defining characteristic of all cells of haematopoietic origin. These share the property that their secretory granules are of lysosomal origin (secretory lysosomes). The mast cells have proved to be the most amenable for experimental manipulation.

Note added in proof

The following reference has been accepted after acceptance of this article: Saito H., Okajima F., Molski T.F.P., Sha'afi R.I., Ui M., Ishizaka T., Effects of ADP-ribosylation of GTP-binding protein by pertussis toxin on immunoglobulin E-dependent and -independent histamine release from mast cells and basophils, J. Immunol. 138 (1987) 3927–3934.

References

[1] Douglas W.W., Rubin R.P., The role of calcium in the secretory response of the adrenal medulla to acetylcholine, J. Physiol. (Lond.) 159 (1961) 40–57.

[2] Douglas W.W., Stimulus-secretion coupling: The concept and clues from chromaffin and other cells, Br. J. Pharmacol. 34 (1968) 451–474.

[3] Dean P.M., Exocytosis modelling: an electrostatic function for calcium in stimulus-secretion coupling, J. Theor. Biol. 54 (1975) 289–308.

[4] Avery J., Jahn R., Edwardson J.M., Reconstitution of regulated exocytosis in cell-free systems: A critical appraisal, Annu. Rev. Physiol. 61 (1999) 777–807.

[5] Baram D., Adachi R., Medalia O., Tuvim M., Dickey B.F., Mekori Y.A., Sagi E.R., Synaptotagmin II negatively regulates Ca^{2+}-triggered exocytosis of lysosomes in mast cells, J. Exp. Med. 189 (1999) 1649–1658.

[6] Baram D., Linial M., Mekori Y.A., Sagi-Eisenberg R., Ca^{2+}-dependent exocytosis in mast cells is stimulated by the Ca^{2+} sensor, synaptotagmin I, J. Immunol. 161 (1998) 5120–5123.

[7] Howell T.W., Cockcroft S., Gomperts B.D., Essential synergy between Ca^{2+} and guanine nucleotides in exocytotic secretion from permeabilized rat mast cells, J. Cell Biol. 105 (1987) 191–197.

[8] Lillie T.H.W., Gomperts B.D., Guanine nucleotide is essential and Ca^{2+} is a modulator in the exocytotic reaction of permeabilized rat mast cells, Biochem. J. 288 (1992) 181–187.

[9] Fernandez J.M., Neher E., Gomperts B.D., Capacitance measurements reveal stepwise fusion events in degranulating mast cells, Nature 312 (1984) 453–455.

[10] Larbi K.Y., Gomperts B.D., Practical considerations regarding the use of streptolysin-O as a permeabilising agent for cells in the investigation of exocytosis, Biosci. Rep. 16 (1996) 11–21.

[11] Barrowman M.M., Cockcroft S., Gomperts B.D., Two roles for guanine nucleotides in the stimulus-secretion sequence of neutrophils, Nature 319 (1986) 504–507.

[12] Metzger H., The receptor with high affinity for IgE, Immunol. Rev. 125 (1992) 37–47.

[13] Park D.J., Min H.K., Rhee S.G., IgE-induced tyrosine phosphorylation of phospholipase C-gamma1 in rat basophilic leukemia cells, J. Biol. Chem. 266 (1991) 24237–24240.

[14] Takai Y., Kishimoto A., Iwasa Y., Kawahara Y., Mori T., Nishizuka Y., Calcium-dependent activation of a multifunctional protein kinase by membrane phospholipids, J. Biol. Chem. 254 (1979) 3692–3695.

[15] Higashijima T., Burnier J., Ross E.M., Regulation of G_i and G_o by mastoparan, related amphiphilic peptides, and hydrophobic amines, J. Biol. Chem. 265 (1990) 14176–14186.

[16] Aridor M., Traub L.M., Sagi-Eisenberg R., Exocytosis in mast cells by basic secretagogues: evidence for direct activation of GTP-binding proteins, J. Cell Biol. 111 (1990) 909–917.

[17] Aridor M., Sagi-Eisenberg R., Neomycin is a potent secretagogue of mast cells that directly activates a GTP-binding protein involved in exocytosis, J. Cell Biol. 111 (1990) 2885–2891.

[18] Foreman J.C., Mongar J.L., Gomperts B.D., Calcium ionophores and movement of calcium ions following the physiological stimulus to a secretory process, Nature 245 (1973) 249–251.

[19] Bennett J.P., Cockcroft S., Gomperts B.D., Ionomycin stimulates mast cell histamine secretion by forming a lipid soluble calcium complex, Nature 282 (1980) 851–853.

[20] Martin M.W., O'Sullivan A.J., Gomperts B.D., Inhibition of cromoglycate and some flavenoids of nucleoside diphosphate kinase and of exocytosis from permeabilized mast cells, Br. J. Pharmacol. 115 (1995) 1080–1086.

[21] Howell T.W., Kramer I.M., Gomperts B.D., Protein phosphorylation and the dependence on Ca^{2+} and GTP-γ-S for exocytosis from permeabilised mast cells, Cell. Signal. 1 (1989) 157–163.

[22] Marquardt D.L., Gruber H.E., Walker L.L., Ribavirin inhibits mast cell mediator release, J. Pharmacol. Exp. Ther. 240 (1987) 145–149.

[23] Wilson B.S., Deanin G.G., Standefer J.C., Vanderjagt D., Oliver J.M., Depletion of guanine nucleotides with mycophenolic acid suppresses IgE receptor-mediated degranulation in rat basophilic leukemia cells, J. Immunol. 143 (1989) 259–265.

[24] Cockcroft S., Howell T., Gomperts B.D., Two G-proteins act in series to control stimulus-secretion coupling in mast cells: use of neomycin to distinguish between G-proteins controlling polyphosphoinositide phosphodiesterase and exocytosis, J. Cell Biol. 105 (1987) 2745–2750.

[25] Aridor M., Rajmilevich G., Beaven M.A., Sagi-Eisenberg R., Activation of exocytosis by the heterotrimeric G protein G_{i3}, Science 262 (1993) 1569–1572.

[26] Mariot P., O' Sullivan A.J., Brown A.M., Tatham P.E.R., Rho-guanine nucleotide dissociation inhibitor protein (Rho-GDI) inhibits exocytosis in mast cells, EMBO J. 15 (1996) 6476–6482.

[27] O' Sullivan A.J., Brown A.M., Freeman H.N., Gomperts B.D., Purification and identification of FOAD-II, a cytosolic protein that regulates secretion in streptolysin-O permeabilised mast cells, as a Rac/RhoGDI complex, Mol. Biol. Cell 7 (1996) 397–408.

[28] Brown A.M., O'Sullivan A.J., Gomperts B.D., Induction of exocytosis from permeabilised mast cells by the guanosine triphosphatases Rac and Cdc42, Mol. Biol. Cell 9 (1998) 1053–1063.

[29] Lamarche N., Tapon N., Stowers L., Burbelo P.D., Aspenstrom P., Bridges T., Chant J., Hall A., Rac and Cdc42 induce actin polymerization and G1 cell cycle progression independently of p65PAK and the JNK/SAPK MAP kinase cascade, Cell 87 (1996) 519–529.

[30] Pinxteren J.A., O'Sullivan A.J., Tatham P.E.R., Gomperts B.D., Regulation of exocytosis from rat peritoneal mast cells by G-protein β subunits, EMBO J. 17 (1998) 6210–6218.

[31] Bar-Sagi D., Gomperts B.D., Stimulation of exocytotic degranulation by microinjection of the ras oncogene protein into mast cells, Oncogene 3 (1988) 463–469.

[32] Oberhauser A.F., Monck J.R., Balch W.E., Fernandez J.M., Exocytotic fusion is activated by Rab3a peptides, Nature 360 (1992) 270–273.

[33] Law G.J., Northrop A.J., Mason W.T., Rab3-peptide stimulates exocytosis from mast cells via a pertussis toxin-sensitive mechanism, FEBS Lett. 333 (1993) 56–60.

[34] Maclean C.M., Law G.J., Edwardson J.M., Stimulation of exocytotic membrane fusion by modified peptides of the rab3 effector domain: re-evaluation of the role of rab3 in regulated exocytosis, Biochem. J. 294 (1993) 325–328.

[35] Smith J., Thompson N., Thompson J., Armstrong J., Hayes B., Crofts A., Squire J., Teahan C., Upton L., Solari R., Rat basophilic leukaemia (RBL) cells overexpressing Rab3a have a reversible block in antigen-stimulated exocytosis, Biochem. J. 323 (1997) 321–328.

[36] Tuvim M.J., Adachi R., Chocano J.F., Moore R.H., Lampert R.M., Zera E., Romero E., Knoll B.J., Dickey B.F., Rab3D, a small GTPase, is localized on mast cell secretory granules and translocates to the plasma membrane upon exocytosis, Am. J. Respir. Cell Mol. Biol. 20 (1999) 79–89.

[37] Fensome A., Cunningham E., Prosser S., Tan S.K., Swigart P., Thomas G., Hsuan J., Cockcroft S., ARF and PITP restore GTP gamma S-stimulated protein secretion from cytosol-depleted HL60 cells by promoting PIP_2 synthesis, Curr. Biol. 6 (1996) 730–738.

[38] Camps M., Hou C., Sidiropoulos D., Stock J.B., Jakobs K.H., Gierschik P., Stimulation of phospholipase C by guanine-nucleotide-binding protein β subunits, Eur. J. Biochem. 206 (1992) 821–831.

[39] Pinxteren J.A., Gomperts B.D., Rogers D., Tatham P.E.R., Thomas G.M.H., Phosphatidyl transfer proteins and protein kinase C make independent contributions to the phosphorylation state necessary for secretory competence in rat mast cells, Abstract 328 at the Keystone Symposium on Protein Kinase C: Structure, Regulation and Cellular Function, Taos, USA, 5-10/2/00.

[40] Marais R.M., Parker P.J., Purification and characterisation of bovine brain protein kinase C isotypes alpha, beta and gamma, Eur. J. Biochem. 182 (1989) 129–137.

[41] Porter F.D., Li Y.S., Deuel T.F., Purification and characterization of a phosphatidylinositol 4-kinase from bovine uteri, J. Biol. Chem. 263 (1988) 8989–8995.

[42] Bazenet C., Brockman J.L., Lewis D., Chan C., Anderson R.A., Erythroid membrane-bound protein kinase binds to a membrane component and is regulated by phosphatidylinositol 4, 5-bisphosphate, J. Biol. Chem. 265 (1990) 7369–7376.

[43] Rao V.D., Misra S., Boronenkov I.V., Anderson R.A., Hurley J.H., Structure of type IIβ phosphatidylinositol phosphate kinase: a protein kinase fold flattened for interfacial phosphorylation, Cell 94 (1998) 829–839.

[44] Stephens L.R., Eguinoa A., Erdjument B.H., Lui M., Cooke F., Coadwell J., Smrcka A.S., Thelen M., Cadwallader K., Tempst P., Hawkins P.T., The G beta gamma sensitivity of a PI3K is dependent upon a tightly associated adaptor, p101, Cell 89 (1997) 105–114.

[45] Billadeau D.D., Brumbaugh K.M., Dick C.J., Schoon R.A., Bustelo X.R., Leibson P.J., The Vav-Rac1 pathway in cytotoxic lymphocytes regulates the generation of cell-mediated killing, J. Exp. Med. 188 (1998) 549–559.

[46] Amoui M., Draberova L., Tolar P., Draber P., Direct interaction of Syk and Lyn protein tyrosine kinases in rat basophilic leukemia cells activated via type I Fc epsilon receptors, Eur. J. Immunol. 27 (1997) 321–328.

[47] Song J.S., Swann P.G., Szallasi Z., Blank U., Blumberg P.M., Rivera J., Tyrosine phosphorylation-dependent and -independent associations of protein kinase C-delta with Src family kinases in the RBL-2H3 mast cell line: regulation of Src family kinase activity by protein kinase C-delta, Oncogene 16 (1998) 3357–3368.

[48] Song J.S., Gomez J., Stancato L.F., Rivera J., Association of a p95 Vav-containing signaling complex with the FcepsilonRI gamma chain in the RBL-2H3 mast cell line. Evidence for a constitutive in vivo association of Vav with Grb2, Raf-1, and ERK2 in an active complex, J. Biol. Chem. 271 (1996) 26962–26970.

[49] Whitehead I.P., Campbell S., Rossman K.L., Der C.J., Dbl family proteins, Biochim. Biophys. Acta 1332 (1997) F1–F23.

[50] Norman J.C., Price L.S., Ridley A.R., Hall A., Koffer A., Actin filament organization in activated mast cells is regulated by heterotrimeric and small GTP-binding proteins, J. Cell Biol. 126 (1994) 1005–1015.

[51] Guo Z.H., Turner C., Castle D., Relocation of the t-SNARE SNAP-23 from lamellipodia-like cell surface projections regulates compound exocytosis in mast cells, Cell 9 (1998) 537–548.

[52] Ting A.T., Schoon R.A., Abraham R.T., Leibson P.J., Interaction between protein kinase C-dependent and G protein-dependent pathways in the regulation of natural killer cell granule exocytosis, J. Biol. Chem. 267 (1992) 23957–23962.

[53] Mittrucker H.W., Fleischer B., Functional localization of an exocytosis-triggering G-protein in human cytotoxic T lymphocytes, Immunology 76 (1992) 610–615.

[54] Coorssen J.R., Davidson M.M., Haslam R.J., Factors affecting dense and alpha-granule secretion from electropermeabilized human platelets: Ca (2+)-independent actions of phorbol ester and GTP gamma S, Cell Regul. 1 (1990) 1027–1041.

[55] Coorssen J.R., Haslam R.J., GTP gamma S and phorbol ester act synergistically to stimulate both Ca (2+)-independent secretion and phospholipase D activity in permeabilized human platelets. Inhibition by BAPTA and analogues, FEBS Lett. 316 (1993) 170–174.

[56] Nüsse O., Lindau M., Cromwell O., Kay A.B., Gomperts B.D., Intracellular application of guanosine-5'-O-(3-thiotriphosphate) induces exocytotic granule fusion in guinea pig eosinophils, J. Exp. Med. 171 (1990) 775–786.

[57] Cromwell O., Bennett J.P., Hide I., Kay A.B., Gomperts B.D., Mechanisms of granule enzyme secretion from permeabilized guinea pig eosinophils, J. Immunol. 147 (1991) 1905–1911.

[58] Larbi K.Y., Gomperts B.D., Complex pattern of inhibition by Mg^{2+} of exocytosis from permeabilised eosinophils, Cell Calcium 21 (1997) 213–219.